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Research Article

Three new endophytic *Apiospora* species (Apiosporaceae, Amphisphaeriales) from China

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Abstract

Apiospora species are widely distributed fungi with diverse lifestyles, primarily functioning as plant pathogens, as well as exhibiting saprophytic and endophytic behaviors. This study reports the discovery of three new species of *Apiospora*, namely *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, isolated from healthy Poaceae plants in China. These novel species were identified through a multi-gene phylogenetic analysis. The phylogenetic analysis of the combined ITS, LSU, *tef1*, and *tub2* sequence data revealed that the three new species formed a robustly supported clade with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Detailed descriptions of the newly discovered species are provided and compared with closely related species to enhance our understanding of the genus *Apiospora*.

Key words: *Apiospora*, Ascomycota, endophyte, phylogeny, taxonomy

Introduction

Apiospora is an important genus of fungal Sordariomycetes, that produces a basauxic, arthrinium-like conidiogenesis (Hyde et al. 2020). The family Apiosporaceae was established to accommodate the genus *Apiospora* with the special conidiogenesis (Hyde et al. 1998). Over time, the membership of Apiosporaceae has undergone several revisions. It presently comprises several genera of fungi with similar morphology, including *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* (Wang et al. 2017; Pintos and Alvarado 2021; Jiang et al. 2022).

Within the family Apiosporaceae, *Apiospora* is closely related to *Arthrinium* and they were once considered as two life stages of a single taxon (Ellis 1965; Crous and Groenewald 2013; Réblová et al. 2016; Jiang et al. 2019). Morphologically, *Apiospora* and *Arthrinium* lack clear diagnostic features, although species of *Arthrinium* often produce conidia of various shapes (Minter and Cannon 2018; Pintos and Alvarado 2021), while most species of *Apiospora* have rounded lenticular conidia (Li et al. 2023; Liao et al. 2023). Ecologically, most sequenced collections of *Arthrinium* were found on Cyperaceae or Juncaceae in temperate, cold, or alpine habitats, while those of *Apiospora* were mainly collected on Poaceae, as well as various other plant host families, in a wide range of habitats, including tropical and subtropical regions (Dai et al. 2016; Jiang et

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al. 2018; Wang et al. 2018; Feng et al. 2021; Tian et al. 2021; Kwon et al. 2022; Monkai et al. 2022). With the addition of molecular evidence and the expansion of the sample, the latest phylogenetic analysis suggests that *Arthrinium* s. str. and *Apiospora* represent independent lineages within Apiosporaceae (Pintos and Alvarado 2021). Consequently, most species of *Arthrinium* have been reclassified under *Apiospora*. Furthermore, Pintos and Alvarado defined the exact identity of *Apiospora montagnei* (the type species of *Apiospora*) and delineated the phylogenetic boundaries of *Apiospora* (Pintos and Alvarado 2022).

Currently, there are 176 records in *Apiospora* (Index Fungorum; http://www.indexfungorum.org/; accessed on 8 Mar 2024). These fungi primarily act as plant pathogens, causing diseases in a wide range of host plants. For example, *A. arundinis* is the causal agent for several important plant diseases, such as kernel blight of barley (Martínez-Cano et al. 1992), brown culm streak of *Phyllostachys praecox* (Chen et al. 2014), moldy sugarcane (Liao et al. 2022), and leaf spot on *Polygonatum cyrtonema* (Gong et al. 2023). *A. marii* causes dieback of olive trees (Gerin et al. 2020), while *A. kogelbergense* leads to blight of *Bambusa intermedi* (Yin et al. 2020). Whereas, many *Apiospora* species are saprophytes, such as *A. acutiapica* (Senanayake et al. 2020), *A. garethjonesii* (Dai et al. 2016), *A. magnispora* (Zhao et al. 2023), *A. sasae* (Crous et al. 2021), and *A. thailandicum* (Dai et al. 2017). In addition, certain *Apiospora* species are reported as endophytes with wide host range, including bamboo (Wang et al. 2018), *Camellia sinensis* (Wang et al. 2018), *Wurfbainia villosa* (Liao et al. 2023), and even hive-stored pollen (Zhao et al. 2018).

Endophytic fungi exhibit rich diversity and play a significant role in the ecosystem. In a previous study, we collected and isolated endophytic fungi from healthy Poaceae plants in China (Liu et al. 2021). In this study, three new endophytic species of *Apiospora* were identified and described based on morphological characteristics and a multi-gene phylogenetic analysis, utilizing a dataset comprising the combined nuclear ribosomal DNA internal transcribed spacer (ITS), nuclear ribosomal DNA large subunit (LSU), the translation elongation factor 1-alpha (*tef1*), and β-tubulin (*tub2*) sequences.

Materials and methods

Fungal isolation

In the present work, Poaceae plant samples were collected from three locations in China: Xilingol Grassland National Nature Reserve in Inner Mongolia, Xishuangbanna, Naban River Watershed National Nature Reserve in Yunnan province, and Baishanzu National Nature Reserve in Zhejiang province (Liu et al. 2021). To isolate endophytic *Apiospora* strains, healthy tissues of asymptomatic plants were first disinfected for 3 min in 75% ethanol and 10 min in 1% sodium hypochlorite, followed by three washes in sterile distilled water. The disinfected tissues were excised, and then incubated on malt extract agar (MEA) medium at 25 °C. Subsequently, the growing hyphae were transferred to potato dextrose agar (PDA) medium to obtain pure cultures.

All strains of *Apiospora* were stored in the Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou, China. In addition, the holotype and

ex-type culture were deposited in the Guangdong Microbial Culture Collection Center (GDMCC). Fungal names were registered in the Fungal Names, one of the recognised repositories of fungal taxonomy (https://nmdc.cn/fungalnames/).

Morphological study

Morphological descriptions were recorded on PDA and MEA. The morphological characteristics of the colonies were captured with a digital camera (Canon EOS700D). The fungal structures were observed and photographed using a stereomicroscope (Leica S9D) and a Leica DM2500 microscope equipped with differential interference contrast (DIC). Measurements of conidiogenous cells and conidia were reported as follows: a-b × c-d (mean, n), where "a" and "c" represent the minimum values, "b" and "d" represent the maximum values, and the mean value and number of measurements (n) are shown in parentheses (Wang et al. 2018).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia from pure cultures grown on PDA at 25 °C for 5–7 d were used for DNA extraction. Genomic DNA was extracted following the method as described in Chi et al. (2009).

Polymerase chain reaction (PCR) amplification was applied to amplify four gene fragments, including ITS, LSU, *tef1*, and *tub2*. The primer pairs were used: ITS1/ITS4 for ITS (White et al. 1990), LR0R/LR5 for LSU (Rehner and Samuels 1995), EF1-728F/EF2 for *tef1* (O'Donnell et al, 1998; Carbone and Kohn 1999), and T1/Bt2b for *tub2* (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). PCR program for ITS amplification was conducted with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The annealing temperatures were adjusted to 56 °C for LSU, *tef1*, and *tub2*.

PCR was performed using a Veriti Thermal Cycler (Waltham, MA, USA). Amplification reactions contained 10 µL of 2× Taq Plus Master Mix II (Vazyme, Nanjing, China), 0.8 µL of each primer (10 µM) (Sunya, Hangzhou, China), 0.8 µL of DNA template, and double-distilled water to reach a total volume of 20 µL. Purification and sequencing of PCR products were performed by Sunya Biotechnology Company (Hangzhou, China). All sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

The quality of obtained sequences was assessed using Chromas v.2.6.6 and the sequences were assembled using SeqMan v.7.1.0. The reference sequences were retrieved from GenBank. All sequences, including the reference sequences, were aligned in batches with MAFFT (Katoh and Standley 2013), manually correcting the resulting alignment by MEGA v.11.0.13 where necessary. A single alignment was made using ITS, LSU, *tef1* region including partial exon 4 and partial exon 5 (the largest exon), *tub2* region including exon 2, exon 3, and partial exon 4. Then phylogenetic analyses were conducted using partial sequences of the above four loci.

Table 1. Species of Apiosporaceae used in the phylogenetic analyses. Notes: Strains in this study are marked in bold. "T" indicates a type culture. NA = not available.

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Apiospora acutiapica	KUMCC 20-0209	*Bambusa bambos*	China	MT946342	MT946338	MT947359	MT947365
Apiospora acutiapica	KUMCC 20-0210 T	*Bambusa bambos*	China	MT946343	MT946339	MT947360	MT947366
Apiospora adinandrae	SAUCC 1282B-1 T	Diseased leaves of *Adinandra glischroloma*	China	OR739431	OR739572	OR753448	OR757128
Apiospora adinandrae	SAUCC 1282B-2	Diseased leaves of *Adinandra glischroloma*	China	OR739432	OR739573	OR753449	OR757129
Apiospora agari	KUC21333, SFC20161014-M18 T	*Agarum cribrosum*	South Korea	MH498520	MH498440	MH544663	MH498478
Apiospora aquatic	MFLU 18-1628, S-642 T	Submerged wood	China	MK828608	MK835806	NA	NA
Apiospora arctoscopi	KUC21331, SFC20200506-M05 T	Eggs of *Arctoscopus japonicus*	South Korea	MH498529	MH498449	MN868918	MH498487
Apiospora arundinis	CBS 124788	Living leaves of *Fagus sylvatica*	Switzerland	KF144885	KF144929	KF145017	KF144975
Apiospora arundinis	LC4951	*Dichotomanthes tristaniicarpa*	China	KY494698	KY494774	KY705097	KY705168
Apiospora aseptata	KUNCC 23-14169 T	Living roots of *Dicranopteris pedata*	China	OR590341	OR590335	OR634949	OR634943
Apiospora aurea	CBS 244.83 T	Air	Spain	AB220251	KF144935	KF145023	KF144981
Apiospora balearica	CBS 145129, AP24118 T	Poaceae plant	Spain	MK014869	MK014836	MK017946	MK017975
Apiospora bambusicola	MFLUCC 20-0144 T	*Schizostachyum brachycladum*	Thailand	MW173030	MW173087	MW183262	NA
Apiospora bawanglingensis	SAUCC BW0444 T	Leaves of *Indocalamus longiauritus*	China	OR739429	OR739570	OR753446	OR757126
Apiospora biserialis	CGMCC 3.20135 T	Bamboo	China	MW481708	MW478885	MW522938	MW522955
Apiospora camelliae-sinensis	CGMCC 3.18333, LC5007 T	*Camellia sinensis*	China	KY494704	KY494780	KY705103	KY705173
Apiospora camelliae-sinensis	LC8181	*Brassica rapa*	China	KY494761	KY494837	KY705157	KY705229
Apiospora cannae	ZHKUCC 22-0139	Leaves of *Canna* sp.	China	OR164902	OR164949	OR166286	OR166322
Apiospora cannae	ZHKUCC 22-0127 T	Leaves of *Canna* sp.	China	OR164901	OR164948	OR166285	OR166321
Apiospora chiangraiense	MFLUCC 21-0053 T	Dead culms of bamboo	Thailand	MZ542520	MZ542524	NA	MZ546409
Apiospora chromolaenae	MFLUCC 17-1505 T	*Chromolaena odorata*	Thailand	MT214342	MT214436	MT235802	NA
Apiospora cordylinae	GUCC 10026	*Cordyline fruticosa*	China	MT040105	NA	MT040126	MT040147
Apiospora cordylinae	GUCC 10027 T	*Cordyline fruticosa*	China	MT040106	NA	MT040127	MT040148
Apiospora coryli	CFCC 58978 T	Dead plant culms of *Corylus yunnanensis*	China	OR125564	OR133586	OR139974	OR139978
Apiospora coryli	CFCC 58979 T	Dead plant culms of *Corylus yunnanensis*	China	OR125565	OR133587	OR139975	OR139979
Apiospora cyclobalanopsidis	CGMCC 3.20136 T	*Cyclobalanopsidis glauca*	China	MW481713	MW478892	MW522945	MW522962
Apiospora cyclobalanopsidis	GZCC 20-0103	*Cyclobalanopsidis glauca*	China	MW481714	MW478893	MW522946	MW522963
Apiospora dendrobii	MFLUCC 14-0152 T	Roots of *Dendrobium harveyanum*	Thailand	MZ463151	MZ463192	NA	NA
Apiospora dematiacea	KUNCC 23-14202 T	Living stems of *Dicranopteris ampla*	China	OR590346	OR590339	OR634953	OR634948
Apiospora descalsii	CBS 145130 T	*Ampelodesmos mauritanicus*	Spain	MK014870	MK014837	MK017947	MK017976
Apiospora dichotomanthi	CGMCC 3.18332, LC4950 T	*Dichotomanthes tristaniicarpa*	China	KY494697	KY494773	KY705096	KY705167
Apiospora dichotomanthi	LC8175	*Dichotomanthes tristaniicarpa*	China	KY494755	KY494831	KY705151	KY705223
Apiospora dicranopteridis	KUNCC23-14171 T	Living stems of *Dicranopteris pedata*	China	OR590342	OR590336	OR634950	OR634944
Apiospora dicranopteridis	KUNCC23-14177	Roots of *Dicranopteris pedata*	China	OR590343	OR590337	OR634951	OR634945
Apiospora dongyingensis	SAUCC 0302 T	Leaves of bamboo	China	OP563375	OP572424	OP573264	OP573270

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Apiospora dongyingensis	SAUCC 0303	Leaves of bamboo	China	OP563374	OP572423	OP573263	OP573269
Apiospora elliptica	ZHKUCC 22-0131 T	Dead stems of unknown plant	China	OR164905	OR164952	OR166284	OR166323
Apiospora elliptica	ZHKUCC 22-0140	Dead stems of unknown plant	China	OR164906	OR164953	NA	OR166324
Apiospora endophytica	ZHKUCC 23-0006 T	Living leaves of *Wurfbainia villosa*	China	OQ587996	OQ587984	OQ586062	OQ586075
Apiospora endophytica	ZHKUCC 23-0007	Living leaves of *Wurfbainia villosa*	China	OQ587997	OQ587985	OQ586063	OQ586076
Apiospora esporlensis	CBS 145136 T	*Phyllostachys aurea*	Spain	MK014878	MK014845	MK017954	MK017983
Apiospora esporlensis	UNIPAMPA010	Living leaves of the Antarctic Hairgrass *Deschampsia antarctica*	Antarctica	MN947641	genome	genome	genome
Apiospora euphorbiae	IMI 285638b	*Bambusa* sp.	Bangladesh	AB220241	AB220335	NA	AB220288
Apiospora fermenti	KUC21288, SFC20140423-M86	Seaweeds	South Korea	MF615230	NA	MH544668	MF615235
Apiospora fermenti	KUC21289 T	Seaweeds	South Korea	MF615226	MF615213	MH544667	MF615231
Apiospora gaoyouensis	CFCC 52301T	*Phragmites australis*	China	MH197124	NA	MH236793	MH236789
Apiospora gaoyouensis	CFCC 52302	*Phragmites australis*	China	MH197125	NA	MH236794	MH236790
Apiospora garethjonesii	GZCC 20-0115	Dead culms of bamboo	China	MW481715	MW478894	MW522947	NA
Apiospora garethjonesii	KUMCC 16-0202, JHB004, HKAS 96289 T	Dead culms of bamboo	China	KY356086	KY356091	NA	NA
Apiospora garethjonesii	SICAUCC 22-0027	Bamboo	China	ON228603	ON228659	NA	ON237651
Apiospora garethjonesii	SICAUCC 22-0028	Bamboo	China	ON228606	ON228662	NA	ON237654
Apiospora gelatinosa	GZAAS 20-0107	Bamboo	China	MW481707	MW478889	MW522942	MW522959
Apiospora gelatinosa	HKAS 11962 T	Bamboo	China	MW481706	MW478888	MW522941	MW522958
Apiospora globosa	KUNCC 23-14210 T	Living stems of *Dicranopteris linearis*	China	OR590347	OR590340	OR634954	NA
Apiospora gongcheniae	**GDMCC 3.1045, YNE00465 T**	**Living stems of *Oryza meyeriana* subsp. *granulata***	**China**	**PP033259**	**PP033102**	**PP034683**	**PP034691**
Apiospora gongcheniae	**YNE00565**	**Living stems of *Oryza meyeriana* subsp. *granulata***	**China**	**PP033260**	**PP033103**	**PP034684**	**PP034692**
Apiospora guangdongensis	ZHKUCC 23-0004 T	Living leaves of *Wurfbainia villosa*	China	OQ587994	OQ587982	OQ586060	OQ586073
Apiospora guangdongensis	ZHKUCC 23-0005	Living leaves of *Wurfbainia villosa*	China	OQ587995	OQ587983	OQ586061	OQ586074
Apiospora guiyangensis	HKAS 102403 T	Dead culms of Poaceae	China	MW240647	MW240577	MW759535	MW775604
Apiospora guiyangensis	KUNCC 22-12539	Poaceae plant	China	OQ029540	OQ029613	OQ186444	OQ186446
Apiospora guizhouensis	CGMCC 3.18334, LC5322 T	Air in karst cave	China	KY494709	KY494785	KY705108	KY705178
Apiospora guizhouensis	LC5318	Air in karst cave	China	KY494708	KY494784	KY705107	KY705177
Apiospora hainanensis	SAUCC 1681 T	Leaves of bamboo	China	OP563373	OP572422	OP573262	OP573268
Apiospora hainanensis	SAUCC 1682	Leaves of bamboo	China	OP563372	OP572421	OP573261	OP573267
Apiospora hispanica	IMI 326877 T	Beach sands	Spain	AB220242	AB220336	NA	AB220289
Apiospora hydei	CBS 114990 T	Culms of *Bambusa tuldoides*	China	KF144890	KF144936	KF145024	KF144982
Apiospora hydei	LC7103	Leaves of bamboo	China	KY494715	KY494791	KY705114	KY705183
Apiospora hyphopodii	JHB003, HKAS 96288	Bamboo	China	KY356088	KY356093	NA	NA
Apiospora hyphopodii	MFLUCC 15-003 T	*Bambusa tuldoides*	Thailand	KR069110	NA	NA	NA
Apiospora hyphopodii	SICAUCC 22-0034	Bamboo	China	ON228605	ON228661	NA	ON237653
Apiospora hysterina	AP12118	*Phyllostachys aurea*	Spain	MK014877	KM014844	MK017953	MK017982
Apiospora hysterina	AP29717	*Phyllostachys aurea*	Spain	MK014875	MK014842	MK017952	MK017981
Apiospora hysterina	ICPM 6889 T	Bamboo	New Zealand	MK014874	MK014841	MK017951	MK017980
Apiospora iberica	CBS 145137, AP10118 T	*Arundo donax*	Portugal	MK014879	MK014846	MK017955	MK017984
Apiospora intestine	CBS 135835	Gut of grasshopper	India	KR011352	MH877577	KR011351	KR011350
Apiospora intestine	MFLUCC 21-0052 T	Dead culms of bamboo	Thailand	MZ542521	MZ542525	MZ546406	MZ546410

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Apiospora italic	CBS 145138, AP221017 T	*Arundo donax*	Italy	MK014880	MK014847	MK017956	MK017985
Apiospora italic	CBS 145139	*Phragmites australis*	Spain	MK014881	MK014848	NA	MK017986
Apiospora jatrophae	CBS 134262, MMI00052 T	Living *Jatropha podagrica*	India	JQ246355	NA	NA	NA
Apiospora jiangxiensis	CGMCC 3.18381, LC4577 T	*Maesa* sp.	China	KY494693	KY494769	KY705092	KY705163
Apiospora jiangxiensis	LC4578	*Camellia sinensis*	China	KY494694	KY494770	KY705093	KY705164
Apiospora kogelbergensis	CBS 113332	*Cannomois virgata*	South Africa	KF144891	KF144937	KF145025	KF144983
Apiospora kogelbergensis	CBS 113333 T	Dead culms of Restionaceae	South Africa	KF144892	KF144938	KF145026	KF144984
Apiospora koreanum	KUC21332, SFC20200506-M06 T	Eggs of *Arctoscopus japonicus*	South Korea	MH498524	MH498444	MH544664	MH498482
Apiospora koreanum	KUC21348	Eggs of *Arctoscopus japonicus*	South Korea	MH498523	NA	MN868927	MH498481
Apiospora lageniformis	KUC21686 T	Culms of *Phyllostachys nigra*	Korea	ON764022	ON787761	ON806626	ON806636
Apiospora lageniformis	KUC21687	Culms of *Phyllostachys nigra*	Korea	ON764023	ON787764	ON806627	ON806637
Apiospora locuta-pollinis	LC11683 T	*Brassica campestris*	China	MF939595	NA	MF939616	MF939622
Apiospora longistroma	MFLUCC 11-0479	Dead culms of bamboo	Thailand	KU940142	KU863130	NA	NA
Apiospora longistroma	MFLUCC11-0481 T	Dead culms of bamboo	Thailand	KU940141	KU863129	NA	NA
Apiospora lophatheri	CFCC 58975 T	Diseased leaves of *Lophatherum gracile*	China	OR125566	OR133588	OR139970	OR139980
Apiospora lophatheri	CFCC 58976 T	Diseased leaves of *Lophatherum gracile*	China	OR125567	OR133589	OR139971	OR139981
Apiospora machili	SAUCC 1175A-4 T	Diseased leaves of *Machilus nanmu* of Machilus nanmu	China	OR739433	OR739574	OR753450	OR757130
Apiospora machili	SAUCC 1175	Diseased leaves of *Machilus nanmu* of Machilus nanmu	China	OQ592560	OQ615289	OQ613333	OQ613307
Apiospora magnispora	ZHKUCC 22-0001 T	Dead stems of *Bambusa textilis*	China	OM728647	OM486971	OM543543	OM543544
Apiospora malaysiana	CBS 102053 T	*Macaranga hullettii*	Malaysia	KF144896	KF144942	KF145030	KF144988
Apiospora marianiae	AP18219 T	Dead stems of *Phleum pratense*	Spain	ON692406	ON692422	ON677180	ON677186
Apiospora marii	CBS 497.90 T	Beach sands	Spain	AB220252	KF144947	KF145035	KF144993
Apiospora marinum	KUC21328, SFC20140423-M02 T	Seaweeds	South Korea	MH498538	MH498458	MH544669	MH498496
Apiospora mediterranea	IMI 326875 T	Air	Spain	AB220243	AB220337	NA	AB220290
Apiospora minutispora	1.70E-042 T	Mountain soils	South Korea	LC517882	NA	LC518889	LC518888
Apiospora montagnei	AP19421	*Arundo micrantha*	Spain	ON692418	ON692425	ON677183	ON677189
Apiospora montagnei	AP301120, CBS 148707, PC:0125164 T	*Arundo micrantha*	Spain	ON692408	ON692424	ON677182	ON677188
Apiospora mori	MFLUCC 20-0181 T	Dead leaves of *Morus australis*	China	MW114313	MW114393	NA	NA
Apiospora mori	NCYUCC 19-0340	Dead leaves of *Morus australis*	China	MW114314	MW114394	NA	NA
Apiospora mukdahanensis	MFLUCC 22-0056 T	Dead leaves of bamboo	Thailand	OP377735	OP377742	NA	NA
Apiospora multiloculata	MFLUCC 21-0023 T	Dead culms of Bambusae	Thailand	OL873137	OL873138	NA	OL874718
Apiospora mytilomorpha	DAOM 214595 T	Dead blades of *Andropogon* sp.	India	KY494685	NA	NA	NA
Apiospora neobambusae	CGMCC 3.18335, LC7106 T	Leaves of bamboo	China	KY494718	KY494794	KY806204	KY705186
Apiospora neobambusae	LC7107	Leaves of bamboo	China	KY494719	KY494795	KY705117	KY705187
Apiospora neobambusae	LC7124	Leaves of bamboo	China	KY494727	KY494803	KY806206	KY705195
Apiospora neochinensis	CFCC 53036 T	*Fargesia qinlingensis*	China	MK819291	NA	MK818545	MK818547
Apiospora neochinensis	CFCC 53037	*Fargesia qinlingensis*	China	MK819292	NA	MK818546	MK818548
Apiospora neogarethjonesii	KUMCC 18-0192, HKAS 102408 T	Dead culms of Bambusae	China	MK070897	MK070898	NA	NA
Apiospora neogongcheniae	**GDMCC 3.1047, YNE01248 T**	**Living stems of Poaceae plant**	**China**	**PP033263**	**PP033106**	**PP034687**	**PP034695**

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Apiospora neogongcheniae	**YNE01260**	**Living stems of Poaceae plant**	**China**	**PP033264**	**PP033107**	**PP034688**	**PP034696**
Apiospora neosubglobosa	JHB 006	Bamboo	China	KY356089	KY356094	NA	NA
Apiospora neosubglobosa	JHB 007^{T}	Bamboo	China	KY356090	KY356095	NA	NA
Apiospora obovata	CGMCC 3.18331, LC4940^{T}	*Lithocarpus* sp.	China	KY494696	KY494772	KY705095	KY705166
Apiospora obovata	LC8177	*Lithocarpus* sp.	China	KY494757	KY494833	KY705153	KY705225
Apiospora oenotherae	CFCC 58972	Diseased leaves of *Oenothera biennis*	China	OR125568	OR133590	OR139972	OR139982
Apiospora oenotherae	LS 395	Diseased leaves of *Oenothera biennis*	China	OR125569	OR133591	OR139973	OR139983
Apiospora ovate	CBS 115042^{T}	*Arundinaria hindsii*	China	KF144903	KF144950	KF145037	KF144995
Apiospora pallidesporae	ZHKUCC 22-0129^{T}	Dead wood of unknown host	China	OR164903	OR164950	NA	NA
Apiospora pallidesporae	ZHKUCC 22-0142	Dead wood of unknown host	China	OR164904	OR164951	NA	NA
Apiospora paragongcheniae	**GDMCC 3.1046, YNE00992^{T}**	**Living stems of Poaceae plant**	**China**	**PP033261**	**PP033104**	**PP034685**	**PP034693**
Apiospora paragongcheniae	**YNE01259**	**Living stems of Poaceae plant**	**China**	**PP033262**	**PP033105**	**PP034686**	**PP034694**
Apiospora paraphaeosperma	MFLUCC 13-0644^{T}	Dead culms of bamboo	Thailand	KX822128	KX822124	NA	NA
Apiospora paraphaeosperma	KUC21488	Culms of bamboo	Korea	ON764024	ON787763	ON806628	ON806638
Apiospora phragmitis	CPC 18900^{T}	*Phragmites australis*	Italy	KF144909	KF144956	KF145043	KF145001
Apiospora phyllostachydis	MFLUCC 18-1101^{T}	*Phyllostachys heteroclada*	China	MK351842	MH368077	MK340918	MK291949
Apiospora piptatheri	CBS 145149, AP4817A^{T}	*Piptatherum miliaceum*	Spain	MK014893	MK014860	MK017969	NA
Apiospora piptatheri	SAUCC BW0455	Diseased leaves of *Indocalamus longiauritus*	China	OR739430	OR739571	OR753447	OR757127
Apiospora pseudomarii	GUCC 10228^{T}	Leaves of *Aristolochia debilis*	China	MT040124	NA	MT040145	MT040166
Apiospora pseudohyphopodii	KUC21680^{T}	Culms of *Phyllostachys pubescens*	Korea	ON764026	ON787765	ON806630	ON806640
Apiospora pseudohyphopodii	KUC21684	Culms of *Phyllostachys pubescens*	Korea	ON764027	ON787766	ON806631	ON806641
Apiospora pseudoparenchymatica	CGMCC 3.18336, LC7234^{T}	Leaves of bamboo	China	KY494743	KY494819	KY705139	KY705211
Apiospora pseudoparenchymatica	LC8173	Leaves of bamboo	China	KY494753	KY494829	KY705149	KY705221
Apiospora pseudorasikravindrae	KUMCC 20-0208^{T}	*Bambusa dolichoclada*	China	MT946344	NA	MT947361	MT947367
Apiospora pseudosinensis	CPC 21546^{T}	Leaves of bamboo	Netherlands	KF144910	KF144957	KF145044	MN868936
Apiospora pseudosinensis	SAUCC 0221	Leaves of bamboo	China	OP563377	OP572426	OP573266	OP573272
Apiospora pseudospegazzinii	CBS 102052^{T}	*Macaranga hullettii*	Malaysia	KF144911	KF144958	KF145045	KF145002
Apiospora pterosperma	CBS 123185	*Machaerina sinclairii*	New Zealand	KF144912	KF144959	NA	KF145003
Apiospora pterosperma	CPC 20193, CBS 134000^{T}	*Lepidosperma gladiatum*	Australia	KF144913	KF144960	KF145046	KF145004
Apiospora pusillispermum	KUC21321^{T}	Seaweeds	South Korea	MH498533	MH498453	MN868930	MH498491
Apiospora pusillispermum	KUC21357	Seaweeds	South Korea	MH498532	NA	MN868931	MH498490
Apiospora qinlingensis	CFCC 52303^{T}	*Fargesia qinlingensis*	China	MH197120	NA	MH236795	MH236791
Apiospora qinlingensis	CFCC 52304	*Fargesia qinlingensis*	China	MH197121	NA	MH236796	MH236792
Apiospora rasikravindrae	LC8179	*Brassica rapa*	China	KY494759	KY494835	KY705155	KY705227
Apiospora rasikravindrae	MFLUCC 21-0051	Dead culms of bamboo	Thailand	MZ542523	MZ542527	MZ546408	MZ546412

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Apiospora sacchari	CBS 372.67	Air	Not mentioned	KF144918	KF144964	KF145049	KF145007
Apiospora sacchari	CBS 664.74	Soils under *Calluna vulgaris*	Netherlands	KF144919	KF144965	KF145050	KF145008
Apiospora saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145051	KF145009
Apiospora saccharicola	CBS 831.71	Not mentioned	Netherlands	KF144922	KF144969	KF145054	KF145012
Apiospora sargassi	KUC21228 T	*Sargassum fulvellum*	South Korea	KT207746	KT207696	MH544677	KT207644
Apiospora sargassi	KUC21232	Seaweeds	South Korea	KT207750	NA	MH544676	KT207648
Apiospora sasae	CPC 38165, CBS 146808 T	Dead culms of *Sasa veitchii*	Netherlands	MW883402	MW883797	MW890104	MW890120
Apiospora septata	CGMCC 3.20134, CS19-8 T	Bamboo	China	MW481711	MW478890	MW522943	MW522960
Apiospora septata	GZCC 20-0109	Bamboo Food	China	MW481712	MW478891	MW522944	MW522961
Apiospora serenensis	IMI 326869 T	Excipients, atmosphere and home dust	Spain	AB220250	AB220344	NA	AB220297
Apiospora setariae	CFCC 54041 T	Decaying culms of *Setaria viridis*	China	MT492004	NA	MW118456	MT497466
Apiospora setariae	MT492005	*Setaria viridis*	China	MT492005	NA	MW118457	MT497467
Apiospora setostroma	KUMCC 19-0217	Dead branches of bamboo	China	MN528012	MN528011	MN527357	NA
Apiospora sichuanensis	HKAS 107008 T	Dead culms of Poaceae	China	MW240648	MW240578	MW759536	MW775605
Apiospora sorghi	URM 93000, URM 7417 T	*Sorghum bicolor*	Brazil	MK371706	NA	NA	MK348526
Apiospora sphaerosperma	CBS 114314	Leaves of *Hordeum vulgare*	Iran	KF144904	KF144951	KF145038	KF144996
Apiospora sphaerosperma	CBS 114315	Leaves of *Hordeum vulgare*	Iran	KF144905	KF144952	KF145039	KF144997
Apiospora stipae	CPC 38101, CBS 146804 T	Dead culms of *Stipa gigantea*	Spain	MW883403	MW883798	MW890082	MW890121
Apiospora subglobosa	MFLUCC 11-0397 T	Dead culms of bamboo	Thailand	KR069112	KR069113	NA	NA
Apiospora subrosea	CGMCC 3.18337, LC7292 T	Leaves of bamboo	China	KY494752	KY494828	KY705148	KY705220
Apiospora subrosea	LC7291	Leaves of bamboo	China	KY494751	KY494827	KY705147	KY705219
Apiospora taeanense	KUC21322T	Seaweeds	South Korea	MH498515	NA	MH544662	MH498473
Apiospora taeanense	KUC21359	Seaweeds	South Korea	MH498513	NA	MN868935	MH498471
Apiospora thailandica	MFLUCC 15-0199	Dead culms of bamboo	Thailand	KU940146	KU863134	NA	NA
Apiospora thailandica	MFLUCC 15-0202 T	Dead culms of bamboo	Thailand	KU940145	KU863133	NA	NA
Apiospora tropica	MFLUCC 21-0056	Dead culms of Bambusoideae	Thailand	OK491657	OK491653	NA	OK560922
Apiospora wurfbainiae	ZHKUCC 23-0008 T	*Wurfbainia villosa*	China	OQ587998	OQ587986	OQ586064	OQ586077
Apiospora wurfbainiae	ZHKUCC 23-0009	*Wurfbainia villosa*	China	OQ587999	OQ587987	OQ586065	OQ586078
Apiospora vietnamensis	IMI 99670 T	*Citrus sinensis*	Vietnam	KX986096	KX986111	NA	KY019466
Apiospora xenocordella	CBS 478.86 T	Soils from roadway	Zimbabwe	KF144925	KF144970	KF145055	KF145013
Apiospora xenocordella	CBS 595.66	Soils	Austria	KF144926	KF144971	NA	NA
Apiospora xishuangbannaensis	KUMCC 21-0695 T	*Rhinolophus pusillus*	China	ON426832	OP363248	OR025969	OR025930
Apiospora xishuangbannaensis	KUMCC 21-0696	*Rhinolophus pusillus*	China	ON426833	OP363249	OR025970	OR025931
Apiospora yunnana	DDQ 00281	*Phyllostachys nigra*	China	KU940148	KU863136	NA	NA
Apiospora yunnana	MFLUCC 15-1002 T	*Phyllostachys nigra*	China	KU940147	KU863135	NA	NA
Apiospora yunnanensis	ZHKUCC 23-0014 T	Dead stems of grass	China	OQ588004	OQ587992	OQ586070	OQ586083
Apiospora yunnanensis	ZHKUCC 23-0015	Dead stems of grass	China	OQ588005	OQ587993	OQ586071	OQ586084
Arthrinium austriacum	GZU 345004	*Carex pendula*	Austria	MW208928	NA	NA	NA
Arthrinium austriacum	GZU 345006	*Carex pendula*	Austria	MW208929	MW208860	NA	NA
Arthrinium caricicola	CBS 145127, AP23518	*Carex ericetorum*	China	MK014871	MK014838	MK017948	MK017977
Arthrinium caricicola	CBS 145903, CPC33297 T	Dead and attached leaves	Germany	MN313782	MN317266	NA	MN313861
Arthrinium crenatum	AG19066, CBS 146353 T	*Carex* sp.	France	MW208931	MW208861	MW221917	MW221923
Arthrinium curvatum	AP25418	Leaves of *Carex* sp.	China	MK014872	MK014839	MK017949	NA
Arthrinium japonicum	IFO 30500	*Carex despalata*	Japan	AB220262	AB220356	NA	AB220309
Arthrinium japonicum	IFO 31098	Leaves of *Carex despalata*	Japan	AB220264	AB220358	NA	AB220311
Arthrinium luzulae	AP7619-3	*Luzula sylvatica*	Spain	MW208937	MW208863	MW221919	MW221925
Arthrinium morthieri	GZU 345043	*Cyperaceae carex*	Austria	MW208938	MW208864	MW221920	MW221926
Arthrinium phaeospermum	AP25619, CBS 146355	Poaceae plant	Norway	MW208943	MW208865	NA	NA

Species	Strain Numbers	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*tef1*	*tub2*
Arthrinium puccinioides	CBS 549.86	*Lepidosperma gladiatum*	Germany	AB220253	AB220347	NA	AB220300
Arthrinium sporophleoides	GZU 345102	*Carex firma*	Austria	MW208944	MW208866	NA	MW221927
Arthrinium sporophleum	AP21118, CBS 145154	Dead leaves of *Juncus* sp.	Spain	MK014898	MK014865	MK017973	MK018001
Nigrospora guilinensis	CGMCC 3.18124, LC 3481 T	*Camellia sinensis*	China	KX985983	KX986113	KY019292	KY019459
Nigrospora guilinensis	LC 7301	Stems of *Nelumbo* sp.	China	KX986063	NA	KY019404	KY019608
Nigrospora hainanensis	CGMCC 3.18129, LC 7030 T	Leaves of *Musa paradisiaca*	China	KX986091	KX986112	KY019415	KY019464
Nigrospora hainanensis	LC 6979	Leaves of *Musa paradisiaca*	China	KX986079	NA	KY019416	KY019586
Nigrospora pyriformis	CGMCC 3.18122, LC 2045 T	*Citrus sinensis*	China	KX985940	KX986100	KY019290	KY019457
Nigrospora pyriformis	LC 2688	*Lindera aggregata*	China	KX985941	NA	KY019297	KY019468
Nigrospora vesicularis	CGMCC 3.18128, LC 7010 T	Leaves of *Musa paradisiaca*	China	KX986088	KX986099	KY019294	KY019463
Nigrospora vesicularis	LC 0322	Unknown host plant	Thailand	KX985939	NA	KY019296	KY019467
Neoarthrinium lithocarpicola	CFCC 54456 T	*Lithocarpus glaber*	China	ON427580	ON427582	NA	ON456914
Neoarthrinium lithocarpicola	CFCC 55883	*Lithocarpus glaber*	China	ON427581	ON427583	NA	ON456915
Neoarthrinium trachycarpi	CFCC 53038	*Trachycarpus fortune*	China	MK301098	NA	MK303396	MK303394
Neoarthrinium trachycarpi	CFCC 53039	*Trachycarpus fortune*	China	MK301099	NA	MK303397	MK303395
Sporocadus trimorphus	CFCC 55171	Rose	China	OK655798	OK560389	OL814555	OM401677
Sporocadus trimorphus	ROC 113	Rose	China	OK655799	OK560390	OL814556	OM401678

The sequences were trimmed and concatenated, and subsequent phylogenetic analyses were performed in PhyloSuite platform (Zhang et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-unlinked) using the BIC criterion. Maximum likelihood (ML) phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under Edge-linked partition model for 5000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model, in which the initial 27% of sampled data were discarded as burn-in. The resulting phylogenetic tree was visualized in FigTree v1.4.3. (http:/tree.bio.ed.ac.uk/software/figtree/) with maximum likelihood bootstrap proportions (MLBP) greater than 70% and Bayesian inference posterior probabilities (BIPP) greater than 0.90, as shown at the nodes. The phylogram was edited in Adobe Illustrator v.27.5 (Adobe Systems Inc., USA). All GenBank accession numbers of sequences used in this study are provided in Table 1.

Results

Phylogeny

The combined ITS, LSU, *tef1*, and *tub2* dataset encompassed 215 strains, including six newly sequenced strains, with *Sporocadus trimorphus* CFCC 55171 and ROC 113 serving as the outgroup taxa, and representative species of *Arthrinium*, *Nigrospora*, and *Neoarthrinium* as the sister groups. The multi-locus sequence dataset comprised 2,081 characters, including gaps, with the following character ranges: ITS (1-352), LSU (353-1149), *tef1* (1150-1775), and *tub2* (1776-2081). The topologies of phylogenetic trees generated by ML and BI analyses were congruent, and the BI tree with MLBP and BIPP is presented in Fig. 1.

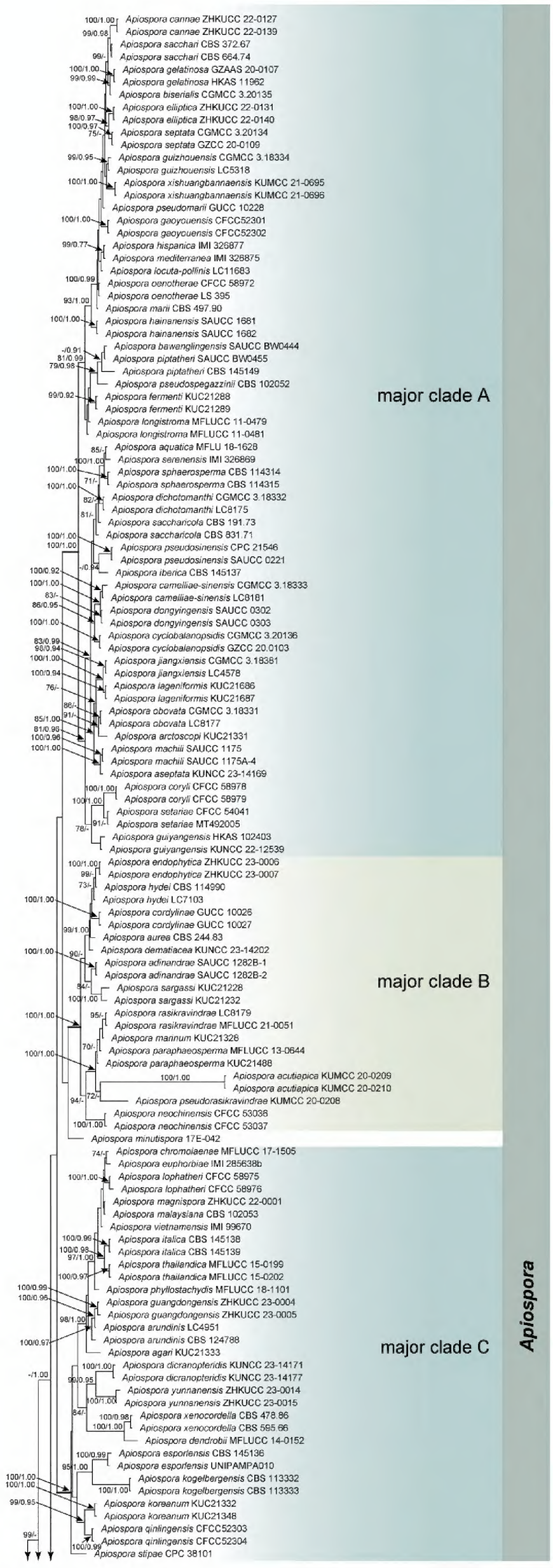

Figure 1. Phylogenetic tree of *Apiospora* based on the combined ITS, LSU, *tef1*, and *tub2* sequences alignment. Maximum likelihood bootstrap proportions ≥70% (left) and Bayesian inference posterior probability ≥0.90 (right) are indicated at nodes (MLBP/BIPP). *Sporocadus trimorphus* (CFCC 55171 and ROC 113) are chosen as the outgroup taxa. The novel species from this study are highlighted in red.

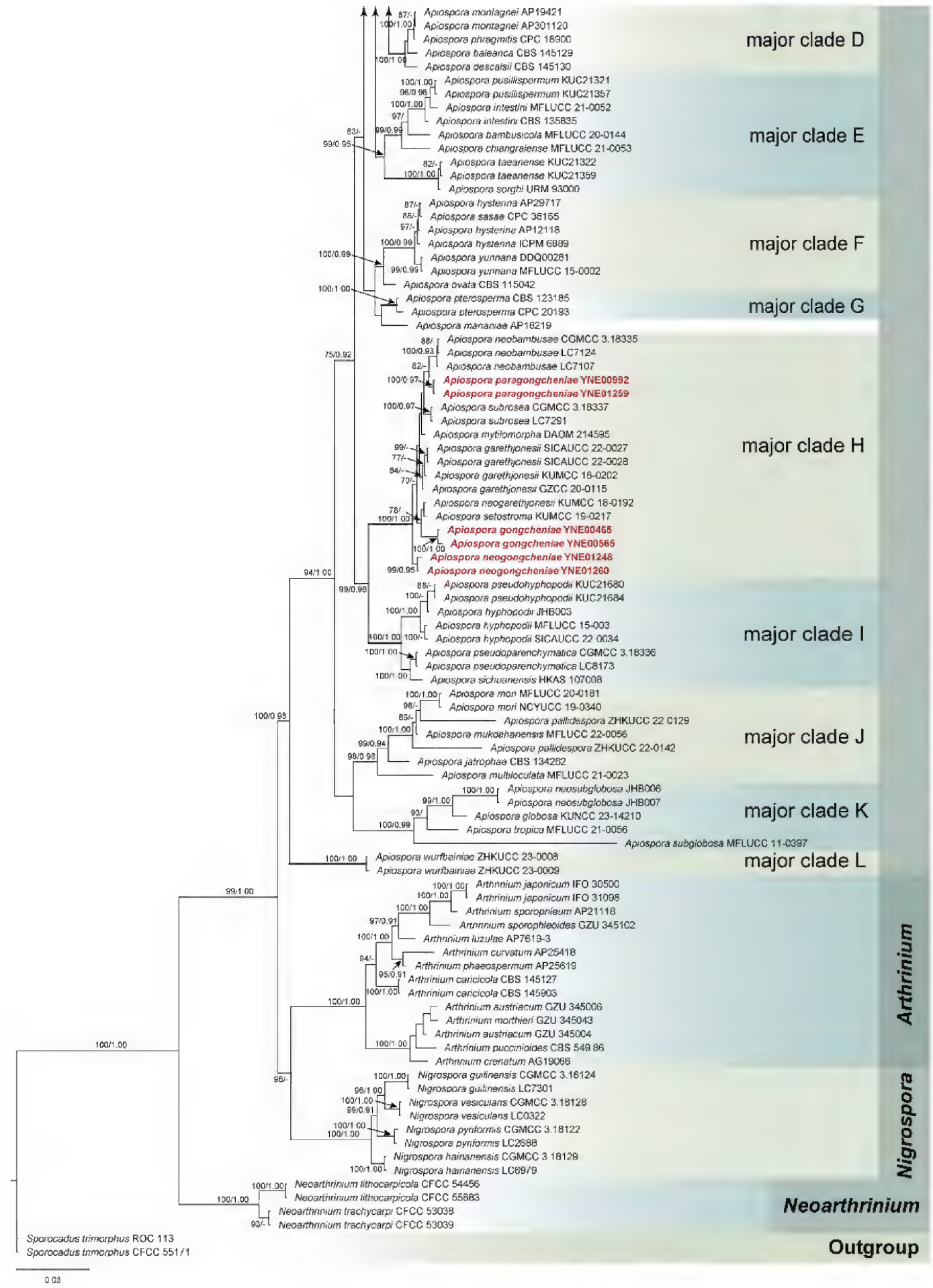

Figure 1. Continued.

The phylogenetic analysis revealed that the species of *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* formed four well-supported distinct lineages. Within the genus *Apiospora*, the 187 strains, encompassing six newly sequenced strains, formed twelve well-supported major clades. The six endophytic strains clustered within one of the major clades H, along with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Concurrently, the six endophytic strains segregated into three independent clades with robust supported values, indicating the presence of three novel species. These novel taxa are formally described herein and assigned the new names *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*.

Taxonomy

***Apiospora gongcheniae* C. L. Zhang, sp. nov.**
Fungal Names: FN 571885
Fig. 2

Etymology. Named after Prof. Gongchen Wang in recognition of her significant contribution to the fields of mycology and plant pathology in China.

Type. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00465 (holotype GDMCC 3.1045, stored in a metabolically inactive state); ex-type culture YNE00465.

Description. ***Asexual morph*:** Hyphae hyaline, branched, septate, smooth, 1.1–2.6 µm diameter (mean = 1.7 µm, n = 30). Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline to pale brown, erect, verrucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, 3.5–9.4 × 1.9–5.2 µm (mean = 5.6 × 3.1 µm, n = 30). Conidia pale brown to dark brown, smooth, granular, globose to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, 8.0–17.0 × 6.8–16.1 µm (mean = 13.6 × 11.6 µm, n = 50). ***Sexual morph*:** Undetermined.

Culture characteristics. On PDA, colonies flat, cottony, dense, margin circular, greyish, reverse light orange, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies dusty pink, dense, covering the 90 mm plate after 7 days at 25 °C. Conidiomata black, globose, abundant, attach to surface of substrate, forming on PDA and MEA after 7–10 days.

Additional specimens examined. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00565.

Note. Phylogenetic analyses confirmed that *A. gongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the GenBank database, it was found that *A. paragongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (93.76% in ITS, 99.81% in LSU), strain GZCC 20-0115 (93.76% in ITS, 99.24% in LSU, 94.06% in *tef1*), strain SICAUCC 22-0027 (93.76% in ITS, 99.81% in LSU, 94.51% in *tub2*), strain SICAUCC 22-0028 (93.76% in ITS, 99.81% in LSU, 93.63% in *tub2*); *A. subrosea* strain CGMCC 3.18337 (96.94% in ITS, 99.42% in LSU, 93.47% in *tef1*, 91.87% in *tub2*), strain LC7291 (90.09% in ITS, 99.41% in LSU, 93.47% in *tef1*, 91.87% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (92.86% in ITS, 99.82% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. gongcheniae*.

As a synopsis of the morphological characteristics presented in Table 2, *A. gongcheniae* differs from *A. garethjonesii* and *A. neogarethjonesii* in having smaller conidia (8.0–17.0 × 6.8–16.1 µm, mean = 13.6 × 11.6 µm) compared to *A. garethjonesii* (surface view: 16–19 µm diam, side view: 17–22 µm diam) and *A. neogarethjonesii* (20–35 × 15–30 µm, mean = 28.5 × 25.6 µm). Additionally, *A. gongcheniae* exhibits shorter conidiogenous cells (3.5–9.4 × 1.9–5.2 µm, mean = 5.6 × 3.1 µm) in contrast to *A. garethjonesii* (6–19 × 3–5 µm,

Table 2. Synopsis of morphological characteristics of related *Apiospora* species. Notes: ND = Not determined.

Strains	***Apiospora garethjonesii*** **(D.Q. Dai & H.B. Jiang) Pintos & P. Alvarado (2021)**	***A. neogarethjonesii*** **(D.Q. Dai & K.D. Hyde) Pintos & P. Alvarado (2021)**	***A. subrosea*** **(M. Wang & L. Cai) Pintos & P. Alvarado (2021)**	***A. neobambusae*** **Pintos & P. Alvarado (2021) (=*Arthrinium bambusae* M. Wang & L. Cai (2018))**	***A. gongcheniae***	***A. paragongcheniae***	***A. neogongcheniae***
Host / Substrate	Dead culms of bamboo	Dead culms of bamboo	Leaves of bamboo	Leaves of bamboo	Stems of *Oryza meyeriana* subsp. *granulata*	Stems of unidentified Poaceae plant	Stems of unidentified Poaceae plant
Known lifestyle	Saprobe	Saprobe	Endophyte	Endophyte	Endophyte	Endophyte	Endophyte
Asci	125–154 × 35–42 µm ($\bar{x}$ = 139 × 38 µm, n = 20), 8-spored	95–125 × 20–25 µm ($\bar{x}$ = 97.6 × 21.3 µm, n = 20), 8-spored	ND	ND	ND	ND	ND
Ascospores	30–42 × 11–16 µm ($\bar{x}$ = 39 × 13 µm, n = 20), 2-seriate, 1-septate, ellipsoidal	25–30 × 9.5–11 µm ($\bar{x}$ = 29.1 × 10.3 µm, n = 20), 2-seriate, overlapping, 1-septate, ellipsoidal, 3–10 µm wide	ND	ND	ND	ND	ND
Conidiomata	Black, with hair-like setae	Black, ellipsoid to irregular, coriaceous	Black, irregular	Black, irregular	Black, globose, abundant, attach to the surface of the substrate	Black, globose to irregular shape, sparse, semi-immersed in the substrate	ND
Conidiophores	Reduced to conidiogenous cells	4.5–6 × 3.5–4.5 µm ($\bar{x}$ = 5.4 × 4.3 µm, n = 20), cylindrical, aseptate	Hyaline to pale brown, smooth, erect or ascending, simple, flexuous, subcylindrical, clustered in groups, aggregated in brown sporodochia, up to 20 µm long, 2–4.5 µm width	Reduced to conidiogenous cells	Reduced to conidiogenous cells	Hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, 12.2–35.1 × 2.1–8.8 µm ($\bar{x}$ = 24.5 × 4.3 µm, n = 30)	ND
Conidiogenous cells	Hyaline to pale brown, smooth, ampulliform, aggregated in black sporodochia, (5–) 6–19 (–20) µm × (2–) 3–5 (–7) µm ($\bar{x}$ = 11 µm × 4 µm, n = 20)	Basauxic, cylindrical, discrete, smooth-walled, 10–48 × 4–5.5 µm ($\bar{x}$ = 35.4 × 4.3 µm, n = 20)	Pale brown, smooth, doliiform to subcylindrical, 3.0–6.5 × 2.0–5.0 µm ($\bar{x}$ = 4.7 ± 1.2 × 3.7 ± 0.9, n = 30)	Hyaline to pale brown, erect, aggregated in clusters on hyphae, smooth, doliiform to ampulliform, or lageniform, 4.0–12.0 × 3.0–7.0 µm ($\bar{x}$ = 6.6 ± 1.8 × 4.8 ± 0.9, n = 30)	Hyaline to pale brown, erect, verrucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, 3.5–9.4 × 1.9–5.2 µm ($\bar{x}$ = 5.6 × 3.1 µm, n = 30)	Hyaline, ampulliform, doliiform to clavate, verrucose, 5.0–13.1 × 2.1–6.0 µm ($\bar{x}$ = 8.2 × 3.9 µm, n = 30)	ND
Conidia	(14–)16–19 (–20) µm diam, brown, smooth, granular, globose to subglobose in surface view, and (16–) 17–22 (–23) µm diam, with pale equatorial slit in side view	Dark brown, globose to subglobose, smooth-walled, with a truncate basal scar, 20–35 × 15–30 µm ($\bar{x}$ = 28.5 × 25.6 µm, n = 20)	Pale brown to dark brown, smooth, globose to subglobose or ellipsoidal, 12.0–17.5 × 9.0–16.0 µm ($\bar{x}$ = 14.9 ± 1.4 × 11.8 ± 1.8, n = 50)	Olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 µm ($\bar{x}$ = 13.2 ± 0.8 × 11.4 ± 1.2, n = 50)	Pale brown to dark brown, smooth, granular, globose to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, 8.0–17.0 × 6.8–16.1 µm ($\bar{x}$ = 13.6 × 11.6 µm, n = 50)	Pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, 8.2–18.7 × 6.4–13.4 µm ($\bar{x}$ = 12.4 × 10.0 µm, n = 50)	ND
References	(Dai et al. 2016; Feng et al. 2021)	(Hyde et al. 2020)	(Wang et al. 2018)	(Wang et al. 2018)	This study	This study	This study

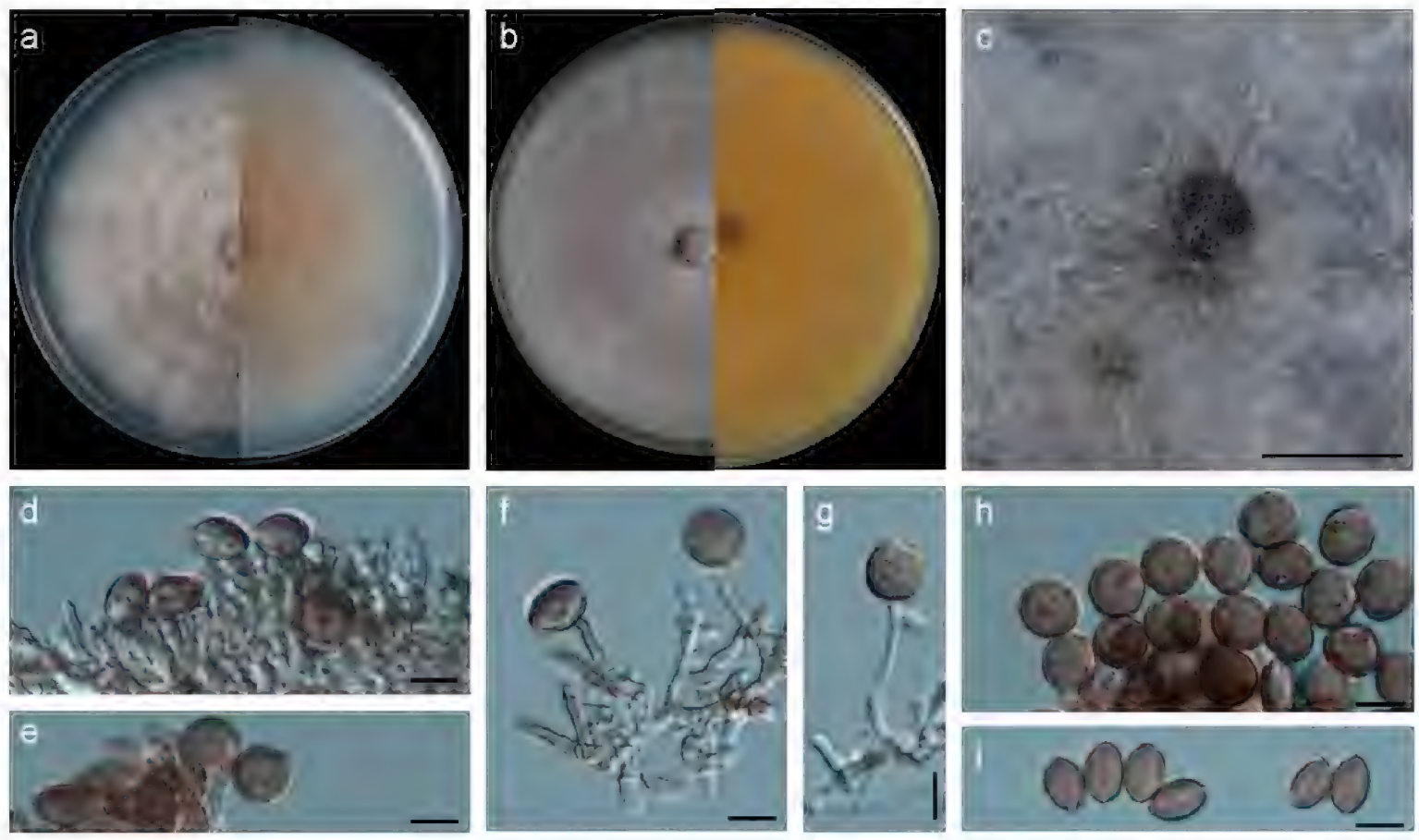

Figure 2. *Apiospora gongcheniae* (YNE00465, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** conidiomata on MEA **d-g** conidiogenous cells giving rise to conidia **h–i** conidia with pale germ slit. Scale bars: 500 μm (**e**); 10 μm (**f–k**).

mean = 11 × 4 μm) and *A. neogarethjonesii* (10–48 × 4–5.5 μm, mean = 35.4 × 4.3 μm). While *A. gongcheniae* shares a similar size range for conidia and conidiogenous cells with *A. subrosea*, it is distinguished by *A. gongcheniae* having conidia featuring a central basal scar and cylindrical conidiogenous cells with tiny denticles. Based on molecular and morphological evidence, we propose *A. gongcheniae* as a new species.

***Apiospora paragongcheniae* C. L. Zhang, sp. nov.**

Fungal Names: FN 571886

Fig. 3

Etymology. Named after its phylogenetic close related to *A. gongcheniae*.

Type. China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of unidentified Poaceae plant, Sep 2016, J.J. Chen, YNE00992 (Holotype GDMCC 3.1046, stored in a metabolically inactive state); ex-type culture YNE00992.

Description. ***Asexual morph*:** Hyphae hyaline, branched, septate, smooth, 1.1–2.2 μm diameter (mean = 1.6 μm, n = 30). Conidiophores hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, 12.2–35.1 × 2.1–8.8 μm (mean = 24.5 × 4.3 μm, n = 30). Conidiogenous cells hyaline, ampulliform, doliiform to clavate, verrucose, 5.0–13.1 × 2.1–6.0 μm (mean = 8.2 × 3.9 μm, n = 30). Conidia pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, 8.2–18.7 × 6.4–13.4 μm (mean = 12.4 × 10.0 μm, n = 50). ***Sexual morph*:** Undetermined.

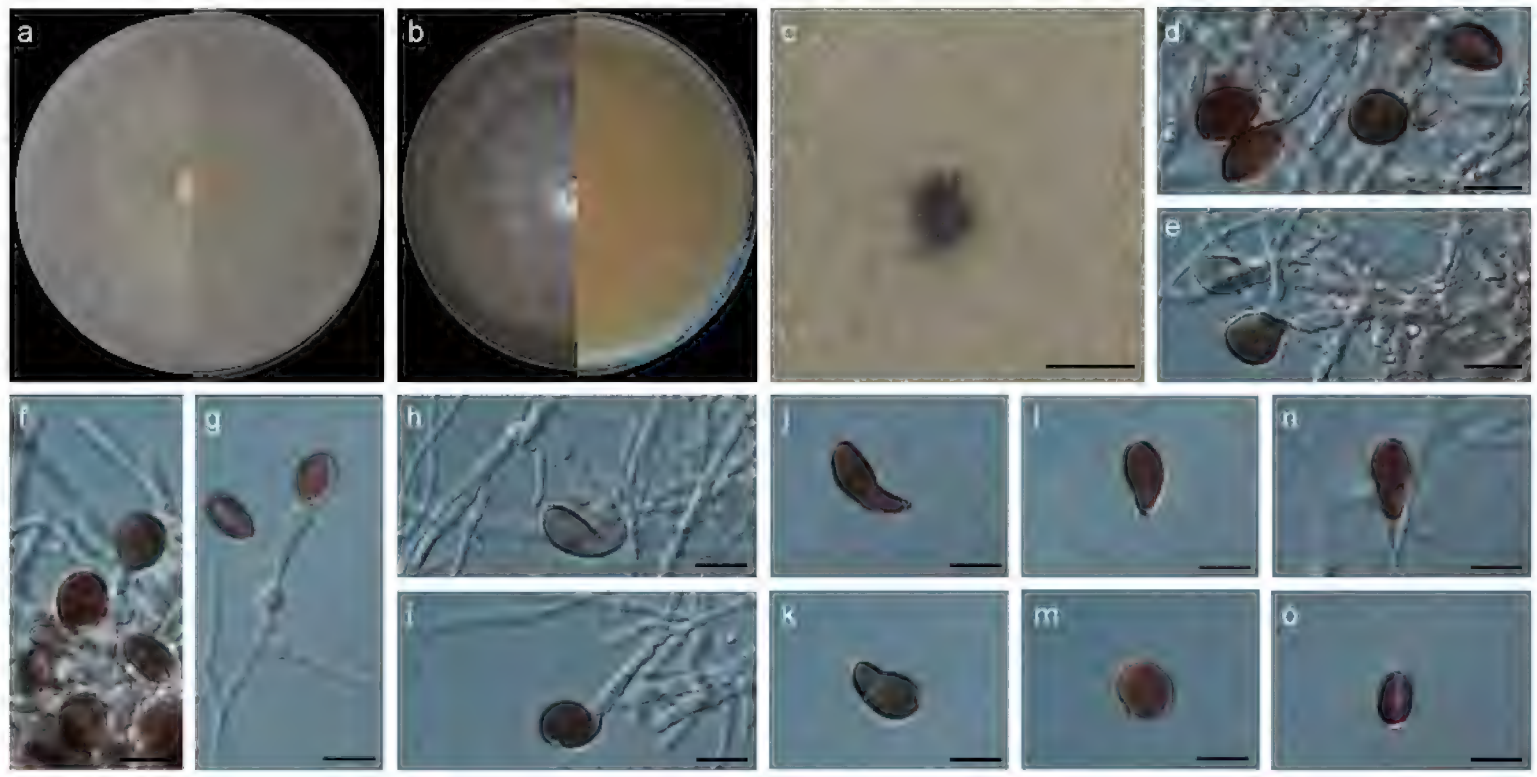

Figure 3. *Apiospora paragongcheniae* (YNE00992, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 6 d at 25 °C on MEA **c** conidioma on MEA **d–i** conidiogenous cells giving rise to conidia **j–o** conidia. Scale bars: 500 µm (**c**); 10 µm (**d–o**).

Culture characteristics. On PDA, colonies flat, rounded, initially white, becoming yellowish-white, with sparse aerial mycelia, mycelium partly immersed in the medium, covering the 90 mm plate after 6 days at 25 °C. On MEA, colonies white, more abundant aerial mycelia, covering the 90 mm plate after 6 days at 25 °C. Conidiomata black, globose to irregular shape, sparse, solitary, semi-immersed in the substrate, observed on MEA after 21–30 days.

Additional specimens examined. China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001259.

Note. Phylogenetic analyses confirmed that *A. paragongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. subrosea*, *A. neobambusae* and *A. neogarethjonesii*. Based on a BLASTN search of the GenBank database, it was found that *A. paragongcheniae* shares high similarities to the following strains: *A. subrosea* strain CGMCC 3.18337 (98.05% in ITS, 99.23% in LSU, 95.93% in *tef1*, 93.63% in *tub2*), strain LC7291 (98.05% in ITS, 99.22% in LSU, 95.93% in *tef1*, 93.63% in *tub2*); *A. neobambusae* strain CGMCC 3.18335 (98.05% in ITS, 100% in LSU, 97.13% in *tef1*, 93.48% in *tub2*), strain LC7107 (98.03% in ITS, 100% in LSU, 94.44% in *tef1*, 93.48% in *tub2*), strain LC7124 (98.05% in ITS, 100% in LSU, 96.82% in *tef1*, 93.47% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (95.43% in ITS, 99.63% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. paragongcheniae*.

As a synopsis of morphological characteristics presented in Table 2, *A. paragongcheniae* distinguishes itself from *A. neobambusae*, *A. neogarethjonesii*, and *A. subrosea* in the shapes and sizes of its conidia. The conidia of *A. paragongcheniae* range from subglobose to oval, occasionally swollen into pyriform to reniform shapes, measuring 8.2–18.7 × 6.4–13.4 µm. This contrasts with *A. neobambusae* (subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 µm), *A. neogarethjonesii* (globose to subglobose, 20–35 × 15–30 µm), and *A. subrosea* (globose to subglobose or ellipsoidal, 12.0–17.5 × 9.0–16.0 µm). Furthermore,

A. paragongcheniae exhibits elongated conidiogenous cells (5.0–13.1 × 2.1–6.0 µm, mean = 8.2 × 3.9 µm) compared to *A. neobambusae* (4.0–12.0 × 3.0–7.0 µm, mean = 6.6 × 4.8 µm) and *A. subrosea* (3.0–6.5 × 2.0–5.0 µm, mean = 4.7 × 3.7 µm). Additionally, *A. paragongcheniae* exhibits shorter conidiogenous cells (5.0–13.1 × 2.1–6.0 µm) compared to *A. neogarethjonesii* (10–48 × 4–5.5 µm). Moreover, these species differ in the morphology of their conidiophores. *A. paragongcheniae* displays hyaline, basauxic, doliiform, subspherical to barrel-shaped conidiophores, whereas *A. neogarethjonesii* has shorter conidiophores, and *A. subrosea* has hyaline to pale brown, simple, subcylindrical conidiophores. Notably, the conidiophores of *A. neobambusae* have reduced to conidiogenous cells.

Apiospora neogongcheniae **C. L. Zhang, sp. nov.**

Fungal Names: FN 571887
Fig. 4

Etymology. Named after its phylogenetic close related to *A. gongcheniae*.

Type. China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE01248 (holotype GDMCC 3.1047, stored in a metabolically inactive state); ex-type culture YNE01248.

Description. ***Asexual morph*****:** Hyphae hyaline, branched, septate, smooth, 1.0–2.5 µm diameter (mean = 1.5 µm, n = 30). Conidia not observed. Chlamydospores single, terminal, globose, rare. ***Sexual morph*****:** Undetermined.

Culture characteristics. On PDA, colonies flat, rounded, initially white, becoming yellowish-white, cottony, with moderate aerial mycelia, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies white, dense aerial mycelia, forming multiple circles around the center, covering the 90 mm plate after 7 days at 25 °C. Conidiomata were not observed.

Additional specimens examined. China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001260.

Note. Phylogenetic analyses confirmed that *A. neogongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the GenBank database, it was found that *A. neogongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (94.88% in ITS, 100% in LSU), strain GZCC 20-0115 (94.88% in ITS, 99.41% in LSU, 96.67% in *tef1*), strain SICAUCC 22-0027 (94.88% in ITS, 100% in LSU, 96.69% in *tub2*), strain SICAUCC 22-0028 (94.88% in ITS, 100% in LSU; 96.79% in *tub2*); *A. subrosea* strain CGMCC 3.18337 (98.35% in ITS, 99.80% in LSU, 94.61% in *tef1*, 94.99% in *tub2*), strain LC7291 (91.41% in ITS, 99.80% in LSU, 94.38% in *tef1*, 94.99% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (93.97% in ITS, 100% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. neogongcheniae*.

Due to the absence of sexual and asexual sporulation characters in *A. neogongcheniae*, a comparison of its culture characteristics with those of *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea* was conducted. On PDA,

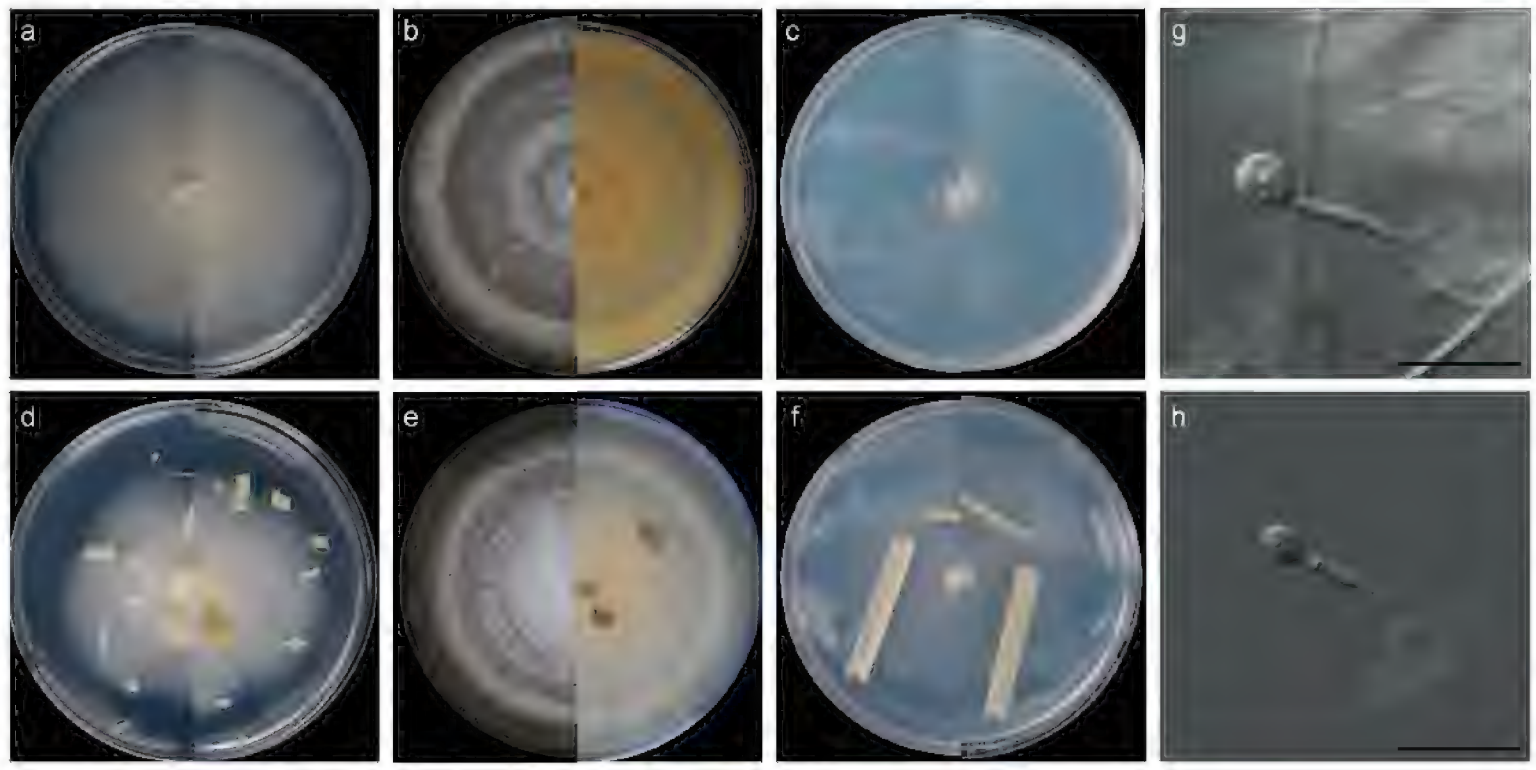

Figure 4. *Apiospora neogongcheniae* (YNE01248, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** colonies after 7 d at 25 °C on SNA **d** colonies after 7 d at 25 °C on PDA with rice leaves **e** colonies after 7 d at 25 °C on MEA with rice leaves **f** colonies after 7 d at 25 °C on SNA with rice leaves **g–h** chlamydospores. Scale bars: 20 μm.

A. neogongcheniae exhibits a yellowish-white surface and reverse color, whereas *A. garethjonesii* displays a white surface with a reddish reverse, *A. neogarethjonesii* shows a white to black surface coloration, and *A. subrosea* presents a light pink surface with a peach-puff reverse. Phylogenetically, *A. neogongcheniae* strains YNE01248 and YNE01260 form a distinct branch with 99% MLBP and 0.95 BIPP. Therefore, we propose *A. neogongcheniae* as a novel species.

Discussion

In the present study, three new species of endophytic *Apiospora* were examined: *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, all of them isolated from the stems of Poaceae plants in Yunnan province of China. According to morphological and molecular identification, the taxonomic position of the three new species was verified.

The generic circumscription of *Apiospora* was primarily defined through phylogenetic analysis, given the limited morphological characteristics of *Apiospora* and *Arthrinium*. The results of a multi-locus phylogenetic analysis in this study, utilizing a combined dataset of ITS, LSU, *tef1*, and *tub2* sequences, supported the previous classification that *Apiospora* and *Arthrinium* are distinct lineages rather than synonyms (Pintos and Alvarado 2021). Unlike the six major clades identified in a previous study (Pintos and Alvarado 2022), the current study revealed twelve major clades with robust support through the phylogenetic analysis of 114 *Apiospora* species, including all known species with available sequences. *Apiospora minutispora* (Das et al. 2020) and *Apiospora marianiae* AP18219 (Pintos and Alvarado 2022) were not classified within these twelve major clades due to their representation by a single record. The delineation of most *Apiospora* species into major clades remained consistent across both studies. Notably, *A. garethjonesii*, *A.neogarethjonesii*, *A. neobambusae*, *A. mytilomorpha*, *A. subrosea*, and *A.setostroma* clustered together in a strongly supported major clade H, aligning with findings from previous studies (Crous et

al. 2021; Monkai et al. 2022; Pintos and Alvarado 2022; Liao et al. 2023; Liu et al. 2024). Within this major clade, three distinct clades representing three new species were identified (Fig. 1). *A. gongcheniae* is distinguished from *A. garethjonesii* by 34/545 nucleotides in the ITS sequences, from *A. neogarethjonesii* by 39/546, and from *A. subrosea* by 13/425. *A. paragongcheniae* is distinguished from *A. subrosea* by 10/512, from *A. neobambusae* by 10/512, and from *A. neogarethjonesii* by 24/525 nucleotides in the ITS sequences. *A. neogongcheniae* is distinguished from *A. garethjonesii* by 28/547, from *A. neogarethjonesii* by 34/547, and from *A. subrosea* by 7/425 nucleotides in the ITS sequences.

Apiospora exhibits ecological diversity, as evidenced by its wide host ranges. Most reported *Apiospora* species show a host preference within the Poaceae family, as noted by Monkai et al. (Monkai et al. 2022). Our new species were also found growing on plant hosts of the Poaceae family. Specifically, *A. gongcheniae* was discovered on the stems of *Oryza meyeriana* subsp. *granulata*, a member of the plant family Poaceae. The other two new species, *A. paragongcheniae* and *A. neogongcheniae*, were found on the stems of unidentified Poaceae plants. Their close relatives, *A. garethjonesii*, *A. neogarethjonesii*, *A. neobambusae*, and *A. subrosea*, were found on bamboo plants. Most *Apiospora* species exhibit saprobic and endophytic lifestyles, which are likely associated with the prevalence of *Apiospora* (Liao et al. 2023). Our new species occurred as endophytic fungi. Further investigation into endophytic *Apiospora* species will significantly enhance the diversity within the *Apiospora* genus.

Morphological characteristics, including asexual and sexual structures, serve as a fundamental basis for fungal systematics and phylogenetic studies, playing a vital role in the comprehensive examination of fungi. However, many endophytes do not form distinct asexual and sexual structures, as observed in *A. neogongcheniae* in this study, posing challenges in determining their taxonomic status based on morphological features. Recent advances in fungal taxonomy and phylogeny have provided new insights into many species with limited morphological features. Future taxonomic efforts necessitate the integration of morphological traits with molecular evidence to elucidate the natural and stable phylogenetic relationships among *Apiospora* species and their related *Arthrinium* species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Xiao-Ni Yan: Investigation, data curation, formal analysis and writing-original draft. Chu-Long Zhang: Conceptualization, methodology, validaiton, formal analysis, supervision, writing-review & editing, funding acquistition.

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Data availability

All of the data that support the findings of this study are available in the main text.

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